Claims

1. Method for analyzing adducts in a fluid and/or solid material suspected of containing said adducts comprising the following steps:

a) bringing said fluid and/or solid material in direct contact with an isothiocyanate reagent containing a fluorescent and, preferably, also an ionizable moiety, with the exception of a reagent in which the isothiocyanate group is directly bound to an unsubstituted phenyl or pentafluorophenyl group;

b) allowing said reagent to react with adducted N-terminals in proteins or peptides present in said fluid and/or solid material;

c) separating the analytes formed from the reaction mixture; and

d) detecting the analytes formed, and optionally visualizing the result.

2. A method according to claim 1 wherein the detection step d) is followed by a step e) comparing the results from the detection step d) with previously obtained results, obtained using steps a) – d), which previously obtained results emanate from a standard material formed from the adduct under scrutiny, and optionally calculating a quotient between said results and optionally presenting said quotient visually.

3. A method according to claim 1 wherein said adducted N-terminals have their adducts attached to a secondary N-terminal valin in hemoglobin, a secondary N-terminal asparagine in serum albumin or to a secondary N-terminal glycine in myoglobin, preferably an N-terminal valin.

4. A method according to claim 1 wherein said adduct is a globin adduct.

5. A method according to claim 1 wherein said adduct is a hemoglobin or a myoglobin adduct.

6. A method according to claim 1 wherein said adduct is a serum albumin adduct.

7. A method according to claim 1 wherein said reagent is a fluorescent compound or a compound that forms fluorescent thiohydantoin analytes.

8. A method according to claim 1 wherein said reagent is an ionizable compound that forms ionizable thiohydantoin analytes.

9. A method according to claim 1 wherein said reagent is a fluorescein compound or a derivative thereof.

10. A method according to claim 1 wherein said reagent is an isothiocyanate reagent containing a fluorescent moiety and an ionizable moiety, preferably selected from the group consisting of 4-isothiocyanato-benzoic acid, 4-isothiocyanato-naphthalene-1-carboxylic acid, 10-isothiocyanato-anthracene-9-carboxylic acid, (4-isothiocyanato-phenyl)-dimethylamine, 9-isothiocyanato-acridine, 4-isothiocyanato-quinoline, malachite green isothiocyanate, FITC, DNITC and DABITC or a derivative thereof; most preferably, FITC, DNITC and DABITC or a derivative thereof; especially most preferably FITC.

11. A method according to claim 1 wherein step c) is performed using LC and/or CE and step d) is performed using LIF and/or fluorescence detection.

12. A method according to claim 1 wherein step c) is performed using CE and/or LC and step d) is performed using UV detection. Preferably, step c) is performed using CE and step d) is performed using a diode array UV detector (DAD).

13. A method according to claim 1 wherein step c) is performed using LC and step d) is performed using MS detection.

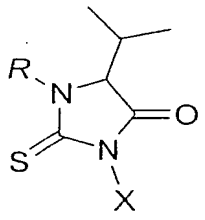
14. A method according to claim 1 wherein step c) is performed using gel electrophoresis and step d) is performed using fluorescence detection.

15. A method according to claim 1 wherein step c) is performed using CE and said CE step is followed by transferring the analyte present onto a rotary device,, preferably a disc, and then step d) is performed using fluorescence detection, which involves illuminating the analyte present and measuring the emitted energy or the absorbed energy, whereby illumination of the analyte present and measurement of the emitted energy or the absorbed energy thereof may be performed an unlimited number of times.

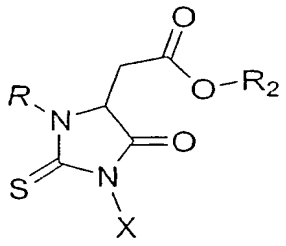
16. A method according to any one of claims 11 to 15 wherein step c) is preceded by a step for enriching the analyte present.

17. A method according to claim 16 wherein said enrichment step preceding step c) is performed using size-discriminating ultrafiltration, preferably followed by an ion-exchanging step, or ultracentrifugation, preferably followed by an ion-exchanging step.

18. A method according to claim 1 wherein said analyte is a compound according to formula I or II, or a derivative thereof:



I XTH-*R*-Val



XTH-*R*-Asp-R_2 II

wherein R represents any adduct (e.g., alkyl and aryl or substituted analogues thereof, with the exception for hydrogen) and X represents a moiety of any isothiocyanate reagent utilized in which the isothiocyanate group is directly bound to an aromatic ring or an aromatic ring system providing fluorescent and/or ionizable properties to the analyte, which the exception that X is not a phenyl, 4-bromophenyl, 4-methoxyphenyl or pentafluorophenyl group, and R_2 represents hydrogen, an alkyl, aryl, carboxyl or benzyl group or substituted analogues thereof; or a carboxyl anion group.

19. A method according to claim 1 wherein detection of the analyte in step d) is performed at a pH above 5, preferably at a pH of approximately 7.

20. A method according to claim 11 or 12 wherein the illumination wavelength in step d) is 488 nm (±20 nm) and the measurement of the emitted energy is performed at longer wavelengths.

21. A method according to claim 1 wherein said fluid and/or solid material is blood or

processed blood, preferably of human origin, which has been obtained at an earlier stage, preferably contained in a container, most preferred a tube.

22. A method according to claim 1 wherein step a) is preceded by obtaining blood from a subject, preferably by cardiac puncture, and collecting said blood in a container, preferably a tube containing anti-coagulant, whereupon the blood is processed; and wherein step b) is followed by heating.

23. A method according to claim 21 or 22 wherein the blood is processed either by centrifugation, washing and lysating, or lysating only.

24. A method according to claim 23 wherein said centrifugation, washing and lysating is followed by heating at approximately 70°C, preferably during approximately 1 h.

25. A method according to claim 23 wherein said lysating only, is followed by heating at approximately 38°C, preferably during approximately 18 h.

26. A method according to claim 22 wherein step b) is performed in a size-discriminating ultra filtration tube, preferably in the outer tube of said ultra filtration tube.

27. A method according to claim 24 wherein the heating is followed by step c) as set out in claim 1 wherein the separation is performed by size-discriminating ultra filtration in a size-discriminating ultra filtration tube and whereupon the analyte is being bound to an ion exchange resin in said tube and thereupon purifying said analyte.

28. A method according to claim 27 wherein the purifying of said analyte is performed by first washing the resin to which the analyte is bound and release the analyte from the resin preferably by adding an acid to said resin, and subsequently filter the resin off giving the analyte in the remaining filtrate.

29. A method according to claim 28 wherein the detecting as set out in step d) of claim 1 is performed by using CE-LIF or LC-MS/MS, preferably LC-MS/MS.

30. A method according to claim 29 wherein alkalization of the detached analytes is performed before detecting using CE-LIF.

31. A method according to claim 25 wherein the heating is followed by step c) as set out in claim 1 wherein the separation is performed by size-discriminating ultra filtration in a size-discriminating ultra filtration tube and wherein the analyte is free in solution and present in the filtrate.

32. A method according to claim 31 wherein the detecting as set out in step d) of claim 1 is performed by using CE-LIF or LC-MS/MS.

33. A method for manufacturing a standard material for use in a method according to any one of claims 1 – 32 consisting of the following steps:

i) reacting an N-substituted amino acid or an adducted N-terminal in a protein or a peptide with a reagent containing a fluorescent and/or ionizable moiety, with the exception of a reagent in which the isothiocyanate group is directly bounded to an unsubstituted phenyl or pentafluorophenyl group; and

ii) purifying the analyte, which is preferably a thiohydantoin analyte formed, by, e.g., separating the unreacted compound from the reaction mixture.

34. A method according to claim 33 wherein said adducted N-terminals have their adducts attached to a secondary N-terminal valin in hemoglobin, a secondary N-terminal asparagine in serum albumin or a secondary N-terminal glycine in myoglobin, preferably an N-terminal valin.

35. A method according to claim 33 wherein said adduct is a globin adduct.

36. A method according to claim 35 wherein said adduct is a hemoglobin or a myoglobin adduct.

37. A method according to claim 33 wherein said adduct is a serum albumin adduct.

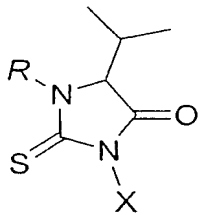
38. A method according to claim 33 wherein said reagent is a fluorescent compound or a compound that forms fluorescent thiohydantoin analytes.

39. A method according to claim 33 wherein said reagent is an ionizable compound that forms ionizable thiohydantoin analytes.

40. A method according to claim 33 wherein said reagent is a fluorescein compound or a derivative thereof.

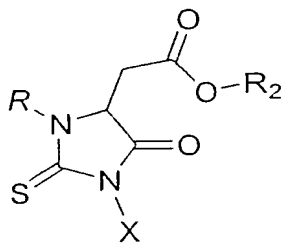
41. A method according to claim 33 wherein said reagent is an isothiocyanate reagent containing a fluorescent moiety and an ionizable moiety, preferably selected from the group consisting of 4-isothiocyanato-benzoic acid, 4-isothiocyanato-naphthalene-1-carboxylic acid, 10-isothiocyanato-anthracene-9-carboxylic acid, (4-isothiocyanato-phenyl)-dimethylamine, 9-isothiocyanato-acridine, 4-isothiocyanato-quinoline, malachite green isothiocyanate, FITC, DNITC and DABITC or a derivative thereof; most preferably, FITC, DNITC and DABITC or a derivative thereof; and especially most preferably FITC.

42. A method according to claim 33 wherein said analyte is a compound according to formula I or II or a derivative thereof;



XTH-*R*-Val

I



XTH-*R*-Asp-R_2

II

wherein R represents any adduct (e.g., alkyl and aryl or substituted analogues thereof, with the exception of hydrogen) and X represents a moiety of any isothiocyanate reagent utilized in which the isothiocyanate group is directly bound to an aromatic ring or an aromatic ring system, thereby providing fluorescent and/or ionizable properties to the analyte, which the exception that X is not a phenyl, 4-bromophenyl, 4-methoxyphenyl or pentafluorophenyl group, and R_2 represents hydrogen; an alkyl, aryl, carboxyl or benzyl moiety or substituted analogues thereof; or a carboxyl anion group.

43. A method according to claim 33 wherein said analyte is a compound selected from the group consisting of 3-[4-(4-dimethylamino-phenylazo)-phenyl]-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DABTH-MeVal); 3-(4-dimethylamino-naphthalen-1-yl)-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DNTH-MeVal); fluorescein, 5-(4-isopropyl-3-methyl-2-thioxo-imidazolidin-5-one) (FTH-MeVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-AAVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-2-hydroxy-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-GAVal); fluorescein, 5-[4-isopropyl-3-(2-hydroxyoctadecyl)-2-thioxo-imidazolidin-5-one] (FTH-HOC_{18}Val); fluorescein, 5-[4-isopropyl-3-(2-hydroxy-propyl)-2-thioxo-imidazolidin-5-one] (FTH-HOPrVal); fluorescein, 5-{4-isopropyl-3-[17-(1,5-dimethyl-hexyl)-3,5 and/or 6-dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-5 and/or 6-yl])-2-thioxo-imidazolidin-5-one} (FTH-CholEOVal) and fluorescein, 5-[4-isopropyl-3-(2,3,4,5,6-pentahydroxy-hexyl)-2-thioxo-imidazolidin-5-one] (FTH-GlcVal).

44. A standard material obtainable by the method according to any one of claims 33 to 43.

45. A compound selected from the group consisting of 3-[4-(4-dimethylamino-phenylazo)-phenyl]-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DABTH-MeVal); 3-(4-dimethylamino-naphthalen-1-yl)-5-isopropyl-1-methyl-2-thioxo-imidazolidin-4-one (DNTH-MeVal); fluorescein, 5-(4-isopropyl-3-methyl-2-thioxo-imidazolidin-5-one) (FTH-MeVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-ethyl)-2-thioxo-imidazolidin-5-one](FTH-AAVal); fluorescein, 5-[4-isopropyl-3-(2-carbamoyl-2-hydroxy-ethyl)-2-thioxo-imidazolidin-5-one] (FTH-GAVal); fluorescein, 5-[4-isopropyl-3-(2-hydroxyoctadecyl)-2-thioxo-imidazolidin-5-one] (FTH-HOC_{18}Val); fluorescein, 5-[4-isopropyl-3-(2-hydroxy-propyl)-2-thioxo-imidazolidin-5-one] (FTH-HOPrVal); fluorescein, 5-{4-isopropyl-3-[17-(1,5-dimethyl-hexyl)-3,5 and/or 6-dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-5 and/or 6-yl])-2-thioxo-imidazolidin-5-one} (FTH-CholEOVal) and fluorescein, 5-[4-isopropyl-3-(2,3,4,5,6-pentahydroxy-hexyl)-2-thioxo-imidazolidin-5-one] (FTH-GlcVal).

46. Use of a standard material according to claim 44 or a compound according to claim 45 in a method according to any one of claims 1 – 32.

47. A container for use when analyzing adducts in a fluid or a solid material

suspected of containing said adducts, wherein said container provides means for performing steps a) – c) as set out in claim 1.

48. Use of a method according to any one of claims 1 – 32 for analyzing hazardous substances, such as acrylamide, glycidamide, styren and aflatoxins.

49. A kit containing standard material according to claim 44 or a compound according to claim 45.

50. A kit containing standard material according to claim 44 or a compound according to claim 45 and a container according to claim 47.

51. An apparatus for performing the method according to any one of claims 1 – 32 and providing means for performing steps a) – c) as set out in claim 1 and for the detection in step d) as set out above.

52. A computer program stored on a data carrier for performing the method according to any one of the claims 1 to 32 or the method according to any one of the claims 33 to 43.